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13. ABSTRACT ***(Maximum 200 Words)***

Radiation therapy is a frequently used treatment for prostate cancer but some prostate cancers respond less well to radiation than others, leading in some cases to recurrence of the cancer. If it could be predicted before treatment whether a patient's prostate cancer was likely to respond well to radiation, then radiation could be given to those likely to respond and be withheld in favor of other treatments in those less likely to have their tumors controlled. Levels of p53, Bcl-2 and epidermal growth factor receptor are being measured in approximately 160 subjects previously treated for prostate cancer, half with radiotherapy and half with radical prostatectomy. These patients were treated greater than 5 years ago and their outcomes are known. The presence or absence of abnormal marker levels in each subject's tumor are being compared to tumor control rates. Relationships between certain markers, the two therapies and control rates may emerge. Such results could identify markers useful in choosing optimal treatment for newly diagnosed prostate cancer patients. Results to date indicate the abnormal p53 status is a very strong, independent predictor of treatment failure, while abnormal bcl-2 status is a less strong but still statistically significant predictor as well.

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INTRODUCTION

Radiation therapy is a primary treatment modality for clinically localized prostate cancer. Laboratory and clinical evidence, however, suggests substantial heterogeneity in the response of prostate cancer to radiation and it is likely that intrinsic differences in cellular radiosensitivity play a major role. Recent attention has focused on the potential of certain molecular determinants to serve as biological response predictors in human cancer. This study is attempting to evaluate the clinical utility of certain candidate markers as specific predictors of prostate cancer response to radiation.

RESULTS

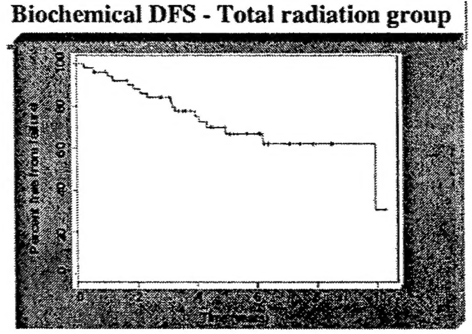
Specific aim:

1) To analyze the clinical outcomes in two rigidly defined groups of patients previously treated with radiotherapy or radical prostatectomy for early stage, favorable-to-intermediate risk prostate cancer. Selected for relatively low pretreatment PSAs and grades, such patients will be likely to have presented with localized disease;

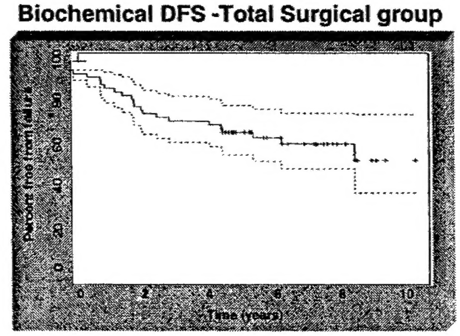
Result: Clinical outcomes (PSA disease free survivals have been determined in approximately 60 patients each who have undergone either radiotherapy or surgery for their early to intermediate stage prostate cancer. The tables of patient characteristics and the actuarial disease free survival curves are shown below. It is noted that disease-free survivals (PSA recurrence free survivals) are similar for the two groups:

Radiation Parameters		Radiation Median (range)	Surgery Parameters		Surgery Median (range)
PSA (ng/ml)			**PSA (ng/ml)**		
Range	# pts.	9.0	Range	# pts.	8.0
1.3 – 4	10		3 – 4	10	
> 4 – 10	21	1.3 - 20	> 4 – 10	29	1.9 – 19.6
>10 – 15	14		>10 – 15	11	
>15 – 20	8		>15 – 20	5	
Gleason score			**Gleason score**		
Range	# pts.	5.5	Range	# pts.	5
≤4	15		≤4	27	
5 - 6	29	3 - 7	5 - 6	18	3 - 8
7	9		7	3	
			8	4	
			N/A	3	
T stage	# pts.	Median F/U	**T stage**	# pts.	Median F/U
T1	21		T1	25	
T2	30	Non-failing pts: 5.1 yrs	T2	22	Non-failing pts: 7.25 yrs
T3	1		T3	1	
N/A	1		N/A	4	

Biochemical DFS - Total radiation group



Biochemical DFS -Total Surgical group

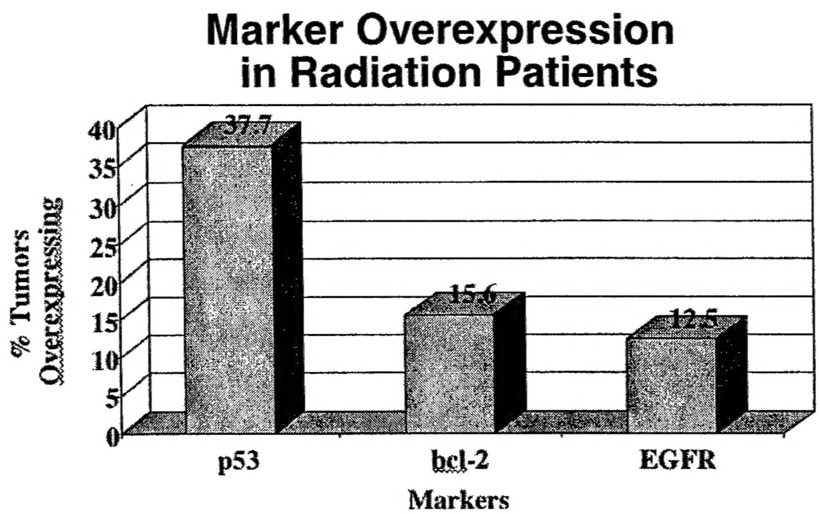


2) To immunohistochemically measure the levels of p53, Bcl-2 and epidermal growth factor receptor (EGFr) in pre-treatment diagnostic biopsy specimens from the same patient cohorts. These markers were selected based upon their prevalence in prostate cancer and their potential linkage to radiation response.

Result: To date, biopsy specimens of 53 patients previously treated with radiation therapy have been analyzed for overexpression of the tumor markers p53, bcl-2 and EGFr. Overexpression of p53 was found to occur in about 38% of radiation therapy patients, whereas bcl-2 abnormal expression only

occurred in about 16%. EGFr was found to be overexpressed in only about 12% of the patients' biopsy samples, rendering it less likely to be useful for predictive purposes.

The radiation studies are being extended to an additional 50 patients and similar studies are currently underway in the surgical cohort (19 patients analyzed for p53 to date, with an abnormal PSA staining percentage of about 30%).



3) To analyze correlations between markers and clinical outcomes in univariate and multivariate fashion, including conventional prognosticators such as stage, grade and PSA;

   Results: It was found that p53 overexpression strongly predicted tumor recurrence in the group of early stage prostate cancer patients treated with radiotherapy Bcl-2 was also found to be predictive of recurrence, although not as strongly as with p53. The p53 marker remained strongly predictive even under multivariate analysis that included such clinically important factors such as grade, stage and pretreatment PSA. Bcl-2, however, did not remain predictive of outcome under multivariate analysis unless p53 status was excluded from the analysis.

   Similar studies are currently underway in the surgical patient cohort.

Multivariate analysis of risk factors for biochemical failure

Risk factor	p value	p value	p value
p53	< 0.0001	-----	< 0.0001
Bcl-2	-----	0.05	0.8
T2/3 (vs. T1)	0.9	0.42	0.9
Gleason (≥7 vs <7)	0.6	0.6	0.7
PSA (>10 vs ≤10)	0.17	0.17	0.2

4) To distinguish between predictors of radioresponse and general prognosticators by comparing marker versus outcome data in the radiotherapy versus surgery patient cohorts.

Result: It will be necessary to complete both the radiotherapy and surgery portions of the study before this comparative analysis can be completed. This analysis will determine whether a given marker such as p53 is specifically predictive for radiation recurrences versus whether it is a global prognosticator that is applicable to surgery-treated patients as well.

KEY RESEARCH ACCOMPLISHMENTS

- Two cohorts of relatively early stage prostate cancer patients, one treated with radiation therapy and the other with surgery, have been identified and have been shown to have similar 5-year disease free survivals after treatment.
- p53 or bcl-2 protein is present in abnormally high amount in a substantial percentage of relatively early stage prostate cancer patients.
- High levels of p53 protein strongly correlates, both under univariate and multivariate analysis with higher rates of subsequent PSA failure in patients treated with radiation therapy.

REPORTABLE OUTCOMES:

- Ritter MA, Gilchrist K, Voytovich M, Verhovan B: p53 as a predictor of radiation response in prostate cancer – early stage disease. Int J Radiat Oncol Biol Phys 53:574-80, 2002.

- "Radiation Therapy Outcomes and p53 Status for Favorable-to-Intermediate Risk Prostate Cancer" 2nd International Meeting on Cancer Diagnostics, NCI-EORTC, June 26-29, 2002.

CONCLUSIONS:

The results of this study to date suggest that at least p53 may be a very strong predictor of outcome after radiotherapy. Given the intrinsic role of p53 in radiation response, there is reason to think that p53 would have such predictive power, but it remains to be determined whether or not this relationship will apply to surgically-treated patients as well.

If pretreatment markers specific for radiation response could be identified and confirmed in additional clinical trials, their availability could ultimately supplement the medical decision-making process and allow a better prospective tailoring of treatment to the biological characteristics of each patient's tumor. For example, a patient predicted to be at high risk for failure specifically after conventional radiotherapy might be better served by surgery or by aggressive dose escalation or perhaps by therapies that targets the identified molecular defect.

REFERENCES: NONE

APPENDIX

Ritter MA, Gilchrist K, Voytovich M, Verhovan B: p53 as a predictor of radiation response in prostate cancer – early stage disease. Int J Radiat Oncol Biol Phys 53:574-80, 2002.

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CLINICAL INVESTIGATION **Prostate**

THE ROLE OF p53 IN RADIATION THERAPY OUTCOMES FOR FAVORABLE-TO-INTERMEDIATE–RISK PROSTATE CANCER

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Purpose: Some prostate cancers may have molecular alterations that render them less responsive to radiation therapy; identification of these alterations before treatment might allow improved treatment optimization. This study investigated whether p53, a potential molecular determinant, could predict long-term radiation therapy outcome in a restricted group of relatively favorable-risk prostate cancer patients treated uniformly with irradiation alone.

Methods and Materials: This study included 53 patients previously treated with radiotherapy for favorable-to-intermediate–risk prostate cancer. These patients were selected for relatively low pretreatment PSAs (≤21 ng/mL) and Gleason scores (≤7) to decrease the likelihood of nonlocalized disease, because disease localization was necessary to examine the efficacy of localized radiation therapy. The status of p53 was immunohistochemically assessed in paraffin-embedded pretreatment biopsy specimens, along with appropriate controls. This marker was selected based upon a usable mutation prevalence in early-stage prostate cancer and its potential linkage with radiation response via cell cycle, DNA repair, and cell death pathways. Correlation between p53 mutation and clinical outcome was analyzed in univariate and multivariate fashion and included conventional prognosticators, such as stage, grade, and PSA. Freedom from biochemical failure was determined using American Society for Therapeutic Radiology and Oncology criteria. Limitations of prior studies were potentially avoided by requiring adequate posttreatment follow-up (median follow-up in nonfailing patients of 5.1 years), as well as pretreatment PSA and Gleason scores that suggested localized disease, and uniformity of treatment.

Results: The total group of 53 favorable-to-intermediate–risk patients demonstrated an actuarial biochemical failure rate of 35% at 5 years. Forty percent of all specimens had a greater than 10% labeling index for p53 mutation, and actuarial biochemical control was found to strongly and independently correlate with p53 status. Patients with higher p53 labeling indices demonstrated significantly higher PSA failure rates ($p < 0.001$). In contrast, p53 status did not correlate with pretreatment PSA, grade, or tumor stage. Similarly, pretreatment PSA (log–rank 0.22), Gleason score (log–rank 0.93), and T stage (log–rank 0.15) were not prognostic for outcome in this group of patients selected for their relatively favorable clinical characteristics.

Conclusions: (*1*) p53 status in pretreatment biopsies strongly predicted for long-term biochemical control after radiation therapy in favorable-to-intermediate–risk prostate cancer patients. (*2*) If validated in other independent clinical data sets, p53 status should be considered as a stratification factor in future clinical trials and could be useful in guiding treatment. Abnormal p53 status might favor surgical management, aggressive dose escalation, or p53-targeted therapy.

Prostatic neoplasms, Radiotherapy, p53, Prognostic factors.

INTRODUCTION

Prostate cancer is the most common nonskin cancer in American men, resulting in more than 30,000 deaths annually in the United States. Despite favorable toxicity profiles and outcomes that may be comparable to those obtained with radical prostatectomy, clinical outcomes after radiation therapy still suggest that local tumor recurrence remains a numerically and clinically important mode of treatment failure (1). Although radiation combined with anti-androgen therapy (2, 3) and conformal dose escalation (4–9) can improve clinical outcome, it would be clinically useful to identify and make prospective use of markers of radiation response.

Pretreatment prostate-specific antigen (PSA), tumor grade, and stage predict for clinical outcome, irrespective of treatment type (10); however, predictors that are specific for radiation response have not been available. Some prostate cancers may have molecular alterations that render them poorly responsive to radiation therapy and that contribute to many of the treatment failures observed after radiation

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therapy. This study investigated whether one such potential molecular determinant, p53, could predict long-term radiation therapy outcome in a selected group of favorable-to-intermediate–risk prostate cancer patients previously treated in uniform fashion with small-field irradiation alone. The choice of p53 was predicated upon its potentially central role in radiation response (11), the existence of some limited clinical correlative studies suggesting that abnormal p53 function predicts for poor radiation therapy outcomes in prostate cancer (12–16), and, lastly, the significant prevalence of p53 mutations in early-stage prostate cancer (12, 13, 16). Furthermore, in the great majority of prostate cancer cases, p53 mutations result in an overaccumulation of functionally inactive p53 protein (17), an accumulation that can be detected using a clinically implementable immunohistochemical approach (18).

This study attempted to minimize potential limitations of several previous studies by requiring adequate posttreatment follow-up (median of 5.1 years in nonfailing patients), uniformity of treatment (no hormonal therapy), and lower pretreatment PSA and Gleason scores, consistent with localized disease. The efficacy of radiation therapy can, of course, be adequately tested only in patients with a high initial likelihood of localized disease. This study's inclusion criteria are clinically relevant in that they mirror the clinical characteristics with which most contemporary prostate cancer patients present.

METHODS AND MATERIALS

Patient selection

A cohort of 67 patients uniformly treated for localized prostate cancer between 1988 and was identified with pretreatment PSAs ≤21 ng/mL, Gleason scores ≤7, and pathology specimens available at our institution. Of these specimens, 14 had insufficient tumor to allow immunohistochemical analysis. The clinical characteristics of the remaining 53 patients (41 needle biopsies and 12 transurethral resections of the prostate) are summarized in Table 1; these patients form the basis for this study. These patients were treated only with small-field radiation therapy, to minimum prostate doses of between 68 and 72 Gy. Because pretreatment PSA and Gleason scores are strong predictors of nonlocalized disease, the selection of patients with relatively favorable values was expected to increase the likelihood of only localized disease at presentation. This condition was necessary to test the efficacy of radiation and the power of certain markers to predict that efficacy. The year 1988 was the earliest for which pretreatment PSAs were routinely available. The selection of early 1995 as a cutoff for eligibility allows for adequate minimum follow-up of clinical outcome. The median follow-up in nonfailing patients was 5.1 years. Clinical outcome was assessed as biochemical (PSA) disease-free survival. PSA failures were defined according to American Society for Therapeutic Radiology and Oncology consensus recommendations (19).

Table 1. Patient characteristics

Parameters	No. of patients (%)	Median	Total range
PSA (ng/mL)		9.0	1.3–21
Range			
3–4	10		
>4–10	21		
>10–15	14		
>15–21	8		
Gleason score		5.5	3–7
Range			
≤4	15		
5–6	29		
7	9		
T stage			
T1	22 (41)		
T2	30 (57)		
T3	1 (2)		

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded blocks from the original diagnostic biopsies. Portions of blocks were sectioned at 5 μm and mounted on slides. One slide from a central section was H&E stained, and the adjacent slides were used for immunohistochemical staining for p53 (clone BP53-12 NeoMarkers, Inc.). Heat-induced epitope retrieval was accomplished using an electric pressure cooker (Decloaking Chamber, BioCare Medical), and slides were stained on an automated immunohistochemistry stainer (Ventana Medical Systems, Inc.). Slides were then lightly counterstained with hematoxylin and scored for the p53 labeling index.

DU-145, PC-3, and PC-3 xenograft tumors were included in staining runs to serve as graded positive and negative controls. The scoring system used is shown in Table 2: By testing, we found that this set of controls, combined with this scoring system, provides scoring consistency over multiple, independent determinations (data not shown).

Statistical analyses

Data were evaluated for disease-specific survival using the Kaplan–Meier product limit method, the log–rank test, and multivariate analysis in a Cox proportional hazards model for markers and other recognized clinical and pathologic predictors of outcome. Deaths due to intercurrent disease were considered losses to follow-up. Predictors were modeled as binary (stage, Gleason score, and PSA) or continuous (p53 score) variables.

Table 2. Immunohistochemistry scoring system for p53

Scoring index	% labeled
0	0
1	1–10
2	11–33
3	34–66
4	67–100

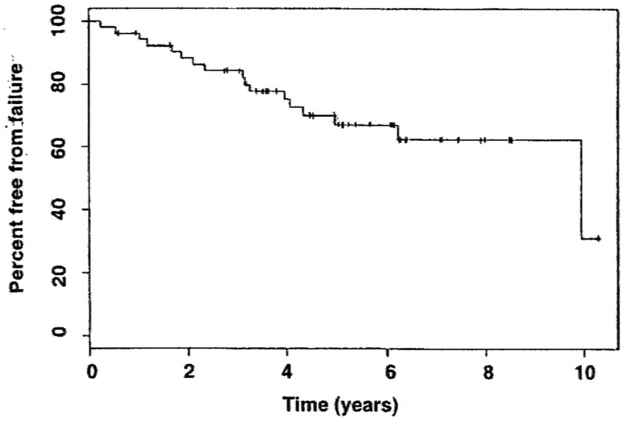

Fig. 1. PSA recurrence-free survival in the total group of 53 patients.

RESULTS

Diagnostic biopsy tissue blocks that were available at our institution and that were from patients meeting the PSA, grade, and treatment date and type eligibility requirements for the study were identified for a group of 53 patients. The clinical outcome of this entire group is shown in Fig. 1, which illustrates an actuarial 35% PSA failure rate at 5 years using American Society for Therapeutic Radiology and Oncology's criteria for PSA failure (19). A total of 17 patients experienced a PSA failure.

p53 analysis

p53 indices were immunohistochemically measured in these 53 previously treated patients, and correlations with standard prognosticators (grade, stage, and PSA) and clinical outcome were analyzed. Twenty patients (37%) had a

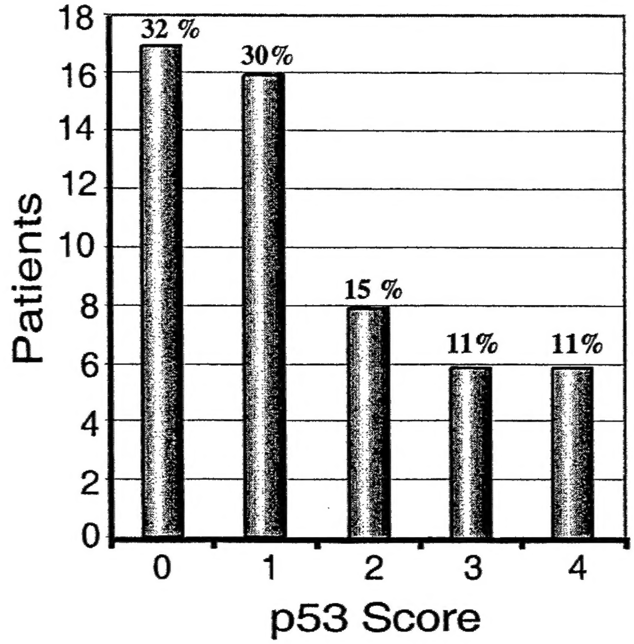

Fig. 2. Distribution of p53 immunohistochemical labeling indices in 53 patients. Score 0 = 0%, score 1 = 1%–10%, score 2 = 11%–33%, score 3 = 34%–66%, and score 4 = 67%–100%.

greater than 10% labeling index (score ≥2), as indicated in Fig. 2.

Low correlation was seen between p53 and pretreatment PSA, grade, or tumor stage, which is the expected result of restricting the PSA, grade, and stage entry criteria for this study. However, clinical outcome measured by PSA control was found to strongly correlate with p53 status. Patients whose tumors demonstrated a greater than 10% p53 labeling index (a scoring index ≥2) demonstrated a 5-year actuarial

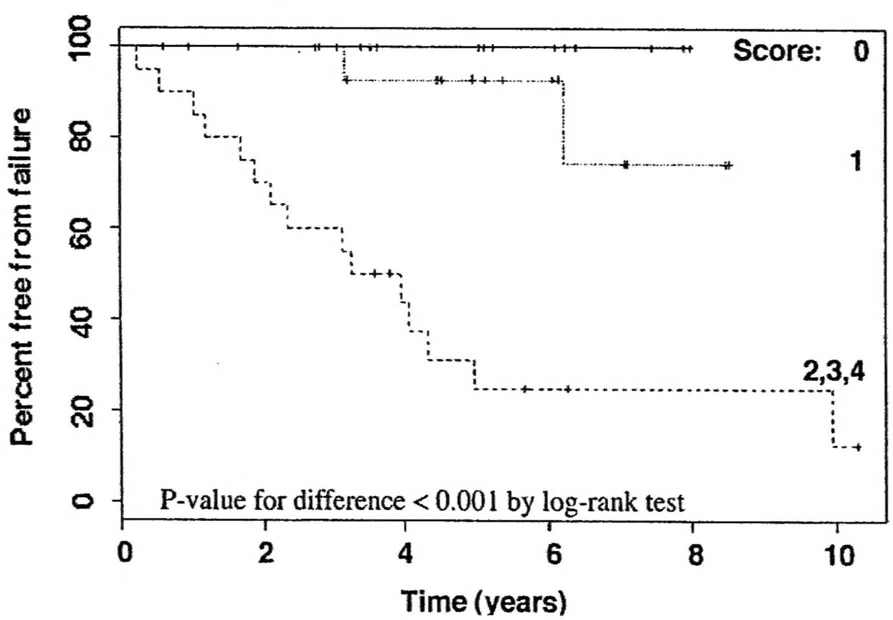

Fig. 3. Biochemical disease-free survival vs. p53 labeling score in 53 patients.

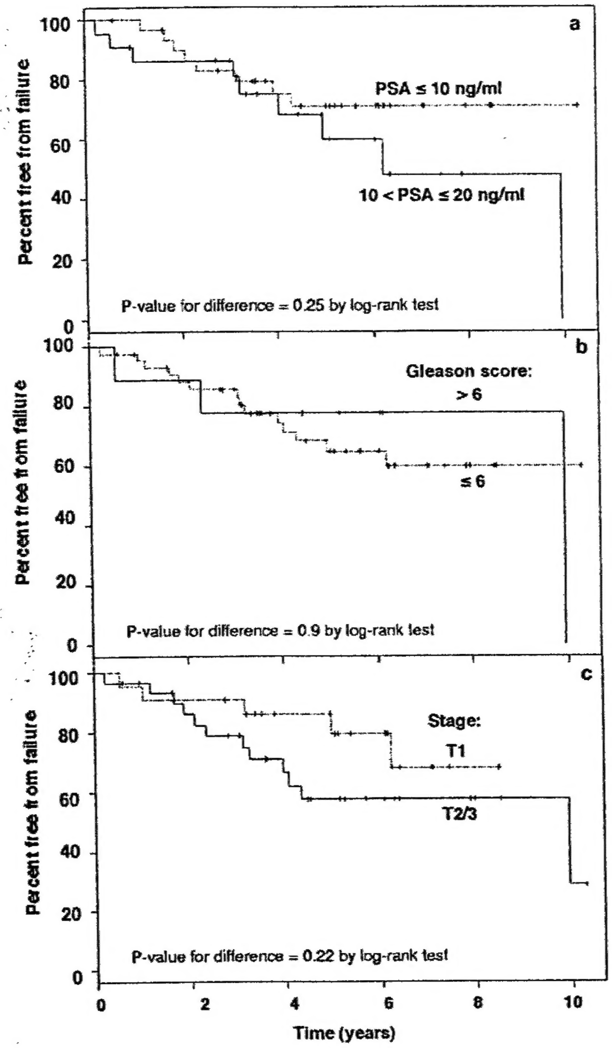

Fig. 4. Biochemical disease-free survival vs. (a) pretreatment PSA, (b) Gleason score, or (c) T stage.

biochemical failure rate of 76% (Fig. 3). In contrast, those patients with p53 labeling indices of 10% or less (score ≤ 1) experienced a biochemical failure rate of only 5% (log–rank, $p < 0.001$). There was a nonsignificant trend toward a somewhat poorer outcome for score 1 vs. score 0 patients with longer follow-up. Of particular note is that no patient with an undetectable p53 labeling index experienced a failure within the follow-up time frame of this study. In fact, a simpler scoring system consisting of zero vs. nonzero p53 labeling (score 0 vs. score ≥ 1) predicted for actuarial estimated 5-year biochemical disease-free survivals of 100% and 54%, respectively ($p = 0.004$).

Pretreatment PSA, Gleason score, or T stage were not prognostic for outcome in this group of patients, having narrowly defined pretreatment characteristics (Fig. 4).

A multivariate analysis was performed that included p53 status, grade, stage, and PSA versus biochemical control. The outcomes of this analysis, including relative risks, are shown in Table 3. It is clear that p53 status is the only variable that had independent prognostic significance in this group of patients. Thus, our results demonstrate a highly significant correlation between poor outcome and high p53 immunostaining.

DISCUSSION

Recent attention has focused on the potential for certain molecular determinants to serve as biologic response predictors in human cancer. Such studies have indicated that the status of biologic markers such as p53, bax, bcl-2, or epidermal growth factor receptor can influence response to radiation in cancers of the breast (20–23), head and neck (24–28), and lung (29, 30). The status of p53 has also been found to alter the *in vitro* (31) and *in vivo* (32) radiation response of prostate cancer cells.

There is also preliminary clinical data for prostate cancer indicating links between radiation response and certain molecular markers. Clinical correlative studies in prostate cancer patients treated with radiation have suggested correlations between outcome and the status of p53, bcl-2, and bax in their tumors (12–16, 33, 34). Markers were determined either in pretreatment biopsies or in material obtained at the time of local tumor recurrence. With one exception (34), immunohistochemically detected abnormal levels of these markers correlated with increased local recurrence. Elevated immunohistochemically detected levels correlated with increased recurrence, except for bax, for which the inverse applied. A summary of these studies is provided in Table 4.

The tumor suppressor gene p53 has been the most extensively studied of these markers in the radiotherapy of prostate cancer, but no studies to date have conclusively linked p53 to radiation response in a clinically useful fashion. Existing studies have provided intriguing clues. However, many studies cited in Table 4 are weakened by low enrollment or the inclusion of patients with

Table 3. Multivariate analysis of clinical and p53 score parameters vs. biochemical control

Risk factor	Hazard ratio	(95% confidence interval)	*p* value
p53 (per unit IHC score increase)	2.3	(1.6, 3.5)	<0.0001
T2/3 (vs. T1)	1.1	(0.3, 3.2)	0.9
Gleason (7 vs. ≤6)	0.7	(0.16, 2.8)	0.6
PSA (>10 vs. ≤10)	2.0	(0.7, 5.5)	0.17

Abbreviation: IHC = immunohistochemistry.

Table 4. Previous studies of p53 and radiotherapy outcomes in prostate cancer

Marker	No. of patients	Predicts failure?	When assessed?	References
p53 Bcl-2	54	+ +	Pretreatment	(12)
p53 GST-pi	55	+ +	At recurrence	(13)
p53	13	+	At recurrence	(14)
Bcl-2 p53	43 pre-RT; 53 post-RT	+ +/−	Both	(33)
Bcl-2	42	−	Pretreatment	(34)
Bcl-2/bax	41	+	Pretreatment	(15)
p53	129	+	Pretreatment	(16)

Abbreviation: RT = radiotherapy.

a broad range of pretreatment prognoses (including very high PSAs, high tumor grades, and even hormonally resistant disease, in some cases) or patients treated with hormonal therapy in addition to radiation. Thus, although the studies suggest predictive, correlative relationships, none conclusively demonstrate predictive capability in the treatment of early-stage prostate cancer with radiation therapy alone.

We attempted to address these issues in our investigation by including only patients with narrowly defined pretreatment characteristics that increased the likelihood of localized disease at the time of treatment. Only patients who had received radiation therapy alone were included. We chose to focus on p53 because of the previous studies suggesting a predictive role for p53 in prostate radiation response and, also importantly, because p53 mutation frequencies are reported to occur in early-stage prostate cancer at an estimated frequency of 20% to 35% (12, 13, 16), sufficiently high enough to make any predictor of outcome clinically useful. Clearly, an infrequently abnormal marker, even if highly correlated with radiosensitivity, would be of little clinical utility.

In addition, there is a strong biologic basis for considering p53 status as a radiation predictor. It has been extensively described as a central mediator of cellular response to DNA-damaging agents, with involvement in induction of the apoptotic response, DNA repair, and cell cycle delay (11). DNA damage induces an increase in p53 protein levels, resulting in the potential activation of numerous molecular pathways. These include transcriptional activation of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$, which potentiates cell cycle arrest (35), as well as activation of GADD45 and its DNA repair–related activities (36). p53 can also induce transcriptional activation of bax (37), thereby promoting apoptosis. Numerous *in vitro* experiments that have manipulated cellular p53 status have found increased resistance to the cytotoxic effects of radiation or chemotherapy when p53 function is disabled (38, 39). Additionally, alterations in p53 function have been shown to reduce cell doubling times (40, 41), a change that might increase tumor clonogen repopulation during multiple-fraction radiation therapy. These findings suggest that dysfunctional p53 will reduce tumor control by radiation. However, because response to ionizing radiation likely involves a number of p53-mediated events that themselves require the integration of both intracellular and extracellular signals, the precise impact of p53 status upon radiosensitivity could vary with, and should be determined in, each type of tumor.

It has been found in the great majority of cases that p53 mutations in prostate cancer result in an overaccumulation of functionally inactive p53 protein (17), which can be detected using an immunohistochemical approach (18). Whereas genomic alterations will certainly be relevant to differential responses to agents such as radiation, the investigation of downstream differences at the protein level takes into account intervening posttranslational processes. Immunohistochemistry remains one of the more clinically practical methods of doing so. Although nonquantitativeness and poor reproducibility can challenge the reliability of this approach, a careful standardization of technique and the use of appropriate, concurrent positive and negative controls can produce a more stable and reliable analysis. Furthermore, as described earlier, we found that even a simplified binary scoring system (zero vs. nonzero p53 labeling) could predict markedly different clinical outcomes.

By design, this study used diagnostic needle biopsies or transurethral resection specimens that can be subject to sampling errors, but such uncertainties apply to virtually all prostate cancer patients treated definitively with radiation therapy. Additionally, although biochemical failure was used as a surrogate for local failure, the entry restrictions in this study were likely to substantially increase the probability that PSA failure reflected local failure.

In conclusion, it was found that p53 status in pretreatment diagnostic specimens strongly predicted for long-term biochemical control after conventional-dose radiation therapy in favorable-to-intermediate–risk prostate cancer patients. The clinical characteristics of patients included in this study are quite similar to those of typical

patients contemporarily diagnosed with prostate cancer. Should these results be further validated in independent data sets, p53 status could be considered as a stratification factor in future clinical trials of low-to-intermediate–risk prostate cancer and could eventually be useful in guiding therapy. An abnormal p53 status might suggest the consideration of surgical management, aggressive radiation dose escalation, or p53-targeted therapy.

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